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Calibration Methods for Field-Flow Fractionation Using Broad Standards. II. Flow Field-Flow Fractionation

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ABSTRACT

Flow field-flow fractionation (FlFFF) is commonly used to determine the molecular weight (MW) of water-soluble polymers. Calibration is usually achieved using monodisperse MW standards, which restricts the determination of absolute MW distribution to a few commonly used polymers. To overcome this limitation, a calibration method using polydisperse standards, which was first developed for thermal field-flow fractionation, has been modified for use in FlFFF. The method was tested using a series of water-soluble polymer samples with known average MW values. The calibration method using polydisperse standards has been validated, and the method should expand the sample range for FlFFF analysis.

INTRODUCTION

Flow field-flow fractionation (FlFFF) is a subtechnique of the field-flow fractionation (FFF) family in which the crossflow of the carrier solvent is used as the external field. To apply the field, the channel walls are made from two porous frits so that the crossflow can be maintained at a right angle to the normal flow (channel flow) down the channel. An appropriate membrane is usually placed over the outlet frit to form the accumulation wall in order to contain the sample molecules within the channel. The crossflow sweeps the sample species toward the accumulation wall where this movement is opposed by the concentration (normal) diffusion process so that a steady-state equilibrium cloud is established. In FlFFF the separation effectively occurs according to differences in the diffusion coefficient which is related to the hydrodynamic diameter of the molecule or particle.

FlFFF is the most universal of all FFF subtechniques. It has been applied to a wide range of samples including virus samples (1), silica particles (2), polystyrene latex beads (3), humic materials (4), and proteins (5). FlFFF has been utilized for characterizing water-soluble polymers since 1978 (6), and a FlFFF apparatus has also been adapted to nonaqueous systems for the separation and characterization of synthetic polymers dissolved in organic solvents (7).

FlFFF can be used to determine the molecular weight distribution (MWD) of a polymer, provided suitable calibration standards are available. Similar to thermal field-flow fractionation (ThFFF), FlFFF calibration is usually based on peak maximum retention for a series of narrow molecular weight (MW) standards of known MW. Narrow MW standards are not available for many polymers, and this strictly limits the utility of FlFFF. Many workers adopt a compromise whereby standards are chosen which are thought to have the same Mark–Houwink constants as the sample polymer. This is equivalent to assuming that the standards and samples have a similar molecular conformation.

In previous and comparison publications, we have developed new calibration methods using broad MW standards for use in ThFFF (8, 9). Because of the similarities between ThFFF and FlFFF techniques, the new calibration methods are also applicable to FlFFF. In this paper the general theory of the calibration method using broad standards for ThFFF is developed for FlFFF. The modified theory was then applied to FlFFF data of some polystyrene sulfonate (PSS) samples to test its validity. The average MW values of the broad standards used in this approach must be independently determined by some other method (e.g., light scattering). Results from this work are presented and discussed.

THEORY

Calibration Using Narrow MW Standards

Sample retention in normal mode FFF separations depends on the mean thickness l of the sample cloud established in response to the applied field driving particles toward the accumulation wall and opposing backdiffusion created by the high sample concentration created at the accumulation wall. The sample cloud thickness l is usually expressed in terms of the dimensionless retention parameter λ as

$$\lambda = l/w \tag{1}$$

where w is the channel thickness.

The retention parameter equation for FlFFF has been derived as (10)

$$\lambda = \frac{DV^0}{\dot{V}_c w^2} \tag{2}$$

where D is the concentration diffusion coefficient, V^0 is the void volume (the retention volume of unretained substances) which is equal to the channel volume, and $\dot{V}_c$ is the crossflow rate. D for a dissolved polymer is related to MW through the empirical equation (11)

$$D = AM^{-b} \tag{3}$$

where A and b are constants describing the molecular conformation of the polymer in the given solvent. If we substitute Eq. (3) for D, Eq. (2) can be expressed as

$$\lambda = \frac{AV^0}{\dot{V}_c w^2 M^b} \tag{4}$$

For all normal mode FFF separations, λ for a given retention is calculated numerically from (12)

$$R = \frac{V^0}{V_r} = 6\lambda \left\{\coth\left(\frac{1}{2\lambda}\right) - 2\lambda\right\} \tag{5}$$

or directly from Eq. (6) for $\lambda \leq 0.15$ ($R \leq 0.63$)

$$\lambda = \frac{3 - (9 - 12R)^{1/2}}{12} \tag{6}$$

For high retention data

$$R = 6\lambda \tag{7}$$

although Eq. (7) is not used in this work

If Eq. (4) is rearranged and the logarithm of both sides is taken, it gives

$$\log\left(\frac{\lambda \dot{V}_c w^2}{V^0}\right) = \log A - b \log M \tag{8}$$

From Eq. (8) it can be seen that a plot of $\log(\lambda \dot{V}_c w^2/V^0)$ (which is equivalent to $\log D$) versus $\log M$ should yield a straight line with intercept $\log A$ and slope $-b$. This linear relationship has been exploited in the conventional calibration of FlFFF for determination of MWD of macromolecules. A major disadvantage of this calibration method in FlFFF is that it requires narrow MW standards of the same or a very similar substance. Only a handful of polymers have such standards available; thus, a calibration method that can utilize broad MW standards should significantly expand on the useful applications of FlFFF. Since FlFFF has certain similarities to ThFFF, the calibration methods developed for ThFFF discussed in Nguyen and Beckett can be readily adapted for FlFFF.

Calibration Using Broad Standards for FlFFF

From Eq. (4) the expression for MW is derived as

$$M = \left(\frac{AV^0}{\dot{v}_c w^2}\right)^{(1/b)} \lambda^{(-1/b)} \tag{9}$$

or

$$M = I_f(\dot{v}_c \lambda)^{-1/b} \tag{10}$$

where

$$I_f = \left(\frac{AV^0}{w^2}\right)^{1/b} \tag{11}$$

General expressions for weight- and number-average MWs (M_w and M_n, respectively) are given by (13)

$$M_w = \frac{\sum_{i=1}^{p} h_i M_i}{\sum_{i=1}^{p} h_i} \tag{12}$$

$$M_n = \frac{\sum_{i=1}^{p} h_i}{\sum_{i=1}^{p} \frac{h_i}{M_i}} \tag{13}$$

where h_i and M_i are the detector signal above the baseline and the corresponding MW respectively of the ith digitized point along the fractogram. p is the total number of digitized points in the fractogram.

Substitution of the expression for M in Eq. (10) into these equations for average MWs yields

$$M_w = \frac{I_f \sum_{i=1}^{p} h_i(\dot{v}_c \lambda_i)^{-1/b}}{\sum_{i=1}^{p} h_i} \tag{14}$$

and

$$M_n = \frac{I_f \sum_{i=1}^{p} h_i}{\sum_{i=1}^{p} \frac{h_i}{(\dot{v}_c \lambda_i)^{-1/b}}} \tag{15}$$

Equation (15) can be rearranged as

$$M_n = \frac{I_f \sum_{i=1}^{p} h_i}{\sum_{i=1}^{p} h_i (\dot{v}_c \lambda_i)^{1/b}} \tag{16}$$

Thus, with a broad MW sample where both M_n and M_w are known or two broad MW samples with either known M_n or M_w values, then constants A and b can be obtained by solving the two Eqs. (14) and (16) using some iterative method such as Newton.

According to the Newton method, b at the qth iteration is calculated from (14)

$$b_{q+1} = b_q - \frac{f_q}{f'_q} \tag{17}$$

where f is a function of b defined differently depending on the combination of average MW used for calibration, and f' is the first derivative of f with respect to b. Once b is obtained, I_f and hence A can be calculated by Eqs. (16) and (11). Function f will be defined specifically for each calibration procedure in the following sections.

Calibration with One Broad Standard Using M_n and M_w

From Eqs. (14) and (15) we have

$$\frac{M_w}{M_n} = \frac{\sum_{i=1}^{p} h_i (\dot{v}_c \lambda_i)^{-1/b} \sum_{i=1}^{p} h_i (\dot{v}_c \lambda_i)^{1/b}}{\left(\sum_{i=1}^{p} h_i\right)^2} \tag{18}$$

Let function f be defined as

$$f = \sum_{i=1}^{p} h_i (\dot{v}_c \lambda_i)^{-1/b} - \frac{A_0}{\sum_{i=1}^{p} h_i (\dot{v}_c \lambda_i)^{-1/b}} \tag{19}$$

where

$$A_0 = \frac{M_w}{M_n}\left(\sum_{i=1}^{p} h_i\right)^2 \tag{20}$$

The first-order derivative f' with respect to b is

$$f' = \left(\frac{1}{b^2}\right)\sum_{i=1}^{p} h_i(\dot{v}_c\lambda_i)^{-1/b}\ln(\dot{v}_c\lambda_i)$$

$$-\frac{A_0}{\left(\sum_{i=1}^{p} h_i(\dot{v}_c\lambda_i)^{1/b}\right)^2}\left\{\left(\frac{1}{b^2}\right)\sum_{i=1}^{p} h_i(\dot{v}_c\lambda_i)^{1/b}\ln(\dot{v}_c\lambda_i)\right\} \tag{21}$$

Calibration with Two Broad Standards Using M_n Values

The expression for M_n for the jth standard, where $j = 1$ or 2, is

$$(M_n)_j = I_f \frac{\sum_{i=1}^{p_j} h_{ji}}{\sum_{i=1}^{p_j} h_{ji}((\dot{v}_c)_j\lambda_{ji})^{1/b}} \tag{22}$$

then

$$\frac{(M_n)_1}{(M_n)_2} = \left\{\frac{\sum_{i=1}^{p_1} h_{1i}}{\sum_{i=1}^{p_1} h_{1i}((\dot{v}_c)_1\lambda_{1i})^{1/b}}\right\}\left\{\frac{\sum_{i=1}^{p_2} h_{2i}((\dot{v}_c)_2\lambda_{2i})^{1/b}}{\sum_{i=1}^{p_2} h_{2i}}\right\} \tag{23}$$

In this case function f is defined by

$$f = \sum_{i=1}^{p_1} h_{1i}((\dot{v}_c)_1\lambda_{1i})^{1/b} - A_0\sum_{i=1}^{p_2} h_{2i}((\dot{v}_c)_2\lambda_{2i})^{1/b} \tag{24}$$

where

$$A_0 = \left(\frac{(M_n)_2}{(M_n)_1}\right)\frac{\sum_{i=1}^{p_1} h_{1i}}{\sum_{i=1}^{p_2} h_{2i}} \tag{25}$$

and the first-order derivative f' is

$$f' = \left(\frac{-1}{b^2}\right) \sum_{i=1}^{p_1} h_{1i}\{(\dot{v}_c)_1\lambda_{1i}\}^{(1/b)} \ln\{(\dot{v}_c)_1\lambda_{1i}\} + \left(\frac{A_0}{b^2}\right) \sum_{i=1}^{p_2} h_{2i}\{(\dot{v}_c)_2\lambda_{2i}\}^{(1/b)} \ln\{(\dot{v}_c)_2\lambda_{2i}\} \tag{26}$$

Calibration with Two Broad Standards Using M_w Values

The expression for M_w for the jth broad standard, for $j = 1$ or 2, is

$$(M_w)_j = I_f \frac{\sum_{i=1}^{p_j} h_{ji}((\dot{v}_c)_j\lambda_{ji})^{-1/b}}{\sum_{i=1}^{p_j} h_{ji}} \tag{27}$$

then

$$\frac{(M_w)_1}{(M_w)_2} = \left\{\frac{\sum_{i=1}^{p_1} h_{1i}((\dot{v}_c)_1\lambda_{1i})^{-1/b}}{\sum_{i=1}^{p_1} h_{1i}}\right\} \left\{\frac{\sum_{i=1}^{p_2} h_{2i}}{\sum_{i=1}^{p_2} h_{2i}((\dot{v}_c)_2\lambda_{2i})^{-1/b}}\right\} \tag{28}$$

As before, f is defined by

$$f = \sum_{i=1}^{p_1} h_{1i}((\dot{v}_c)_1\lambda_{1i})^{-1/b} - A_0 \sum_{i=1}^{p_2} h_{2i}((\dot{v}_c)_2\lambda_{2i})^{-1/b} \tag{29}$$

but in this case

$$A_0 = \frac{(M_w)_1}{(M_w)_2} \left(\frac{\sum_{i=1}^{p_1} h_{1i}}{\sum_{i=1}^{p_2} h_{2i}}\right) \tag{30}$$

and the first-order derivative f' is

$$f' = \left(\frac{1}{b^2}\right) \sum_{i=1}^{p_1} h_{1i}\{(\dot{v}_c)_1\lambda_{1i}\}^{(-1/b)} \ln\{(\dot{v}_c)_1\lambda_{1i}\} - \left(\frac{A_0}{b^2}\right) \sum_{i=1}^{p_2} h_{2i}\{(\dot{v}_c)_2\lambda_{2i}\}^{(-1/b)} \ln\{(\dot{v}_c)_2\lambda_{2i}\} \tag{31}$$

Calibration with Multiple Broad Standards Using M_w Values

Assuming there are m broad standards for calibration, a new function $\mathbf{f}(I_f,b)$ for the jth standard, where $j = 1, 2, \ldots, m$, is defined by

$$\mathbf{f}_j = \mathbf{f}_j(I_f,b) = \left\{ (M_\mathrm{w})_j - \frac{I_f \sum_{i=1}^{p_j} h_{j,i}((\dot{v}_\mathrm{c})_j \lambda_{ji})^{-1/b}}{\sum_{i=1}^{p_j} h_{ji}} \right\}^2 \tag{32}$$

To determine the values of constants I_f and b for FlFFF, we adopt the same process as described in Nguyen and Beckett but replace S_j (or ΔT_j) by $\dot{v}_c$. The optimization method returns the values of I_f and b. A is then calculated from Eq. (11).

EXPERIMENTAL

Instrumentation

The FlFFF channel (supplied by FFFractionation Inc., Utah, USA) was the same as described in Beckett et al. (4). It contained a cellulose acetate membrane supported by alumina ceramic frits. The channel had a tip-to-tip length of 27.2 cm, a channel thickness of 0.0254 cm, and a breadth of 2.04 cm, resulting in a geometric void volume of 1.28 mL. The volume of tubing from the outlet of the channel to the inlet of the UV detector (dead volume) was measured to be 0.51 mL.

A fluid delivery module model F-4000 (FFFractionation Inc., Utah, USA) multihead piston pump was used for generating the field and channel flows and as an unpump for controlling the fluid flow rate from the crossflow stream. Samples were introduced to the channel through a 20-μL Rheodyne 6-way injection valve. A BAS model UV-8 UV absorbance detector was used at a wavelength of 254 nm. Flow rates were measured by two in-house microprocessor flowmeters, each connected to an electronic balance, one to measure the channel flow rate (balance model PT200 from Mettler) and one to measure the crossflow rate (balance model FX-300 from AND). A chart recorder model no. DP600 from ICI Instruments was used to monitor data from the detector. Carrier solution contained 0.05 M tris(hydroxymethyl)methylamine and 3.08×10^{-3} M NaN_3. HNO_3 was added to adjust the pH of the solution at 8.

Run Conditions and Data Manipulation

The FlFFF fractograms were recorded by the chart recorder. They were later digitized using a Hewlett-Packard plotter with the GRAPHPAD software package and stored in floppy diskettes for later processing. The program FFF.EXE supplied by FFFractionation Inc. (Utah, USA) was used to adjust the baseline and remove the void peak for these fractograms. Subsequent data analysis was carried using in-house programs written in GWBASIC.

Materials

Narrow MW Standards. PSS narrow MW standards (PSS1–PSS4) were obtained from Polymer Standards Service (Mainz, Germany). The MW values supplied by the manufacturer and the FlFFF run conditions for these standards are given in Table 1.

Broad MW Samples. Four broad MW polysulfonated polysaccharide (PSPS) samples (PSPS1–PSPS4), obtained from the Institute of Drug Technology (Boronia, Victoria, Australia), were used for testing the computational programs as well as for the calibration procedures. Since MW data for these samples were not available, average MW and polydispersity values for use in the calculations were generated based on the PSS standards on the assumption that they have molecular conformations similar to that of the PSS standards. The MW so obtained and the FlFFF run conditions for these samples are given in Table 2.

RESULTS AND DISCUSSIONS

The narrowly dispersed PSS standards were run under conditions of field (crossflow) and channel flow rates indicated in Table 1. The fractograms of these narrow standards are shown in Fig. 1. The retention ratio (R) data measured at the peak maximum of the fractograms are also summarized in Table 1. The corresponding retention parameters at the peak maximum were calculated using either Eq. (5) or (6). These data

TABLE 1

MW Values (M_p) Supplied by the Manufacturer, FlFFF Run Conditions, and Retention Data at the Peak Maximum Obtained for the Four PSS Standards

Standard number	M_p (dalton)	Channel flow rate (mL/min)	Crossflow rate (mL/min)	V_r (mL)	R	λ
PSS1	1,370	4.33	4.31	4.54	0.281	0.0523
PSS2	4,800	4.48	4.27	8.45	0.151	0.0266
PSS3	6,200	4.45	4.22	10.80	0.118	0.0205
PSS4	18,500	4.35	4.25	18.70	0.068	0.0116

TABLE 2

FlFFF Run Conditions, MW Values, and Polydispersity for the Four PSPS Samples. The MW Average and Polydispersity Values Were Calculated Using the Fractograms in Fig. 3 and the Constants *A* and *b* Obtained from the Calibration Line Shown in Fig. 2

Sample number	Channel flow rate (mL/min)	Crossflow rate (mL/min)	M_n (dalton)	M_w (dalton)	$\mu(M_w/M_n)$
PSPS1	2.96	2.81	5700	19,200	3.4
PSPS2	2.92	2.80	3500	10,600	3.0
PSPS3	2.96	2.83	2000	4,900	2.4
PSPS4	2.91	2.83	6600	16,700	2.5

and the manufacturer's peak maximum MW (M_p) were used to plot the calibration line shown in Fig. 2. The slope of this line is $b = 0.587$, and the y-intercept $\log A = -2.102$, which gave a value of 7.921×10^{-3} for A.

These constants (A and b) were used to backcalculate the MW of each of the standards; these MW values are summarized in Table 6. Good agreement was found between M_p values obtained from the FlFFF calibration line and those specified by the manufacturer (up to 12% deviation). Note that the manufacturer's M_p values were actually the MW at the SEC

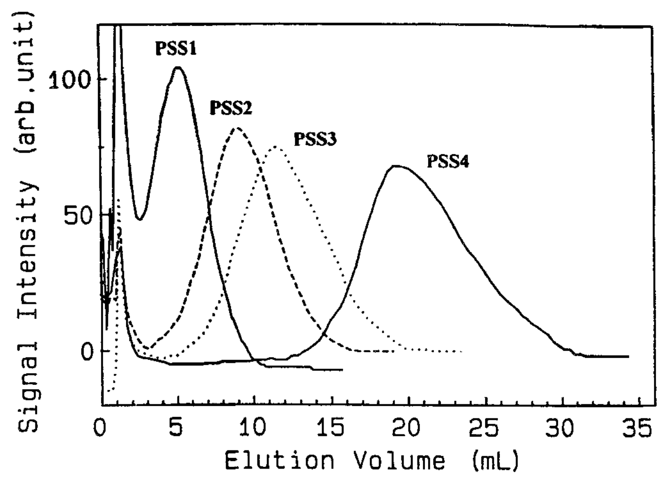

FIG. 1 Fractograms for four PSS standards, run at conditions indicated in Table 1.

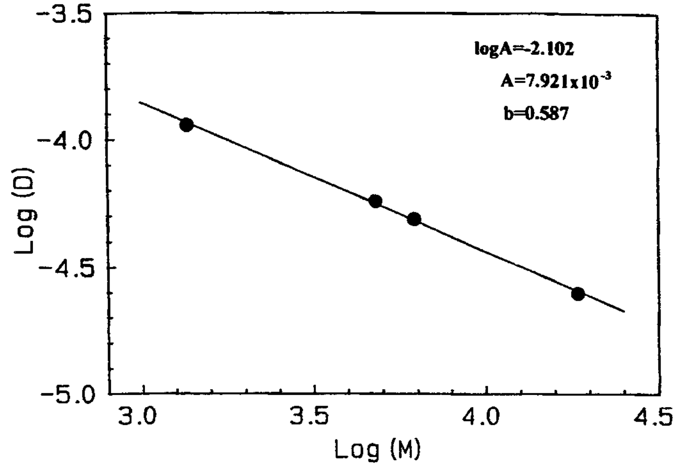

FIG. 2 Calibration line constructed using FlFFF peak maximum data indicated in Table 1 for four PSS standards. Crossflow and channel flow rates used in FlFFF runs for these standards are also given in Table 1.

peak maximum rather than for the FlFFF fractogram. The backcalculated M_n and M_w values had some larger deviations from the manufacturer's given values (up to 21% for M_n and up to 24% for M_w) than the deviations recorded for the M_p values, no doubt because calibration was achieved using the M_p data rather than M_w or M_n.

Fractograms of the four broad PSPS samples are given in Fig. 3. The constants A and b obtained from the PSS calibration line in Fig. 2 were then used to generate MWD for these samples. These MWD curves are shown in Fig. 4. MW averages and polydispersity for each sample are tabulated in Table 2. These MW values will be used later to test the proposed calibration method for polydisperse samples.

The digitized fractograms of the broad MW PSPS samples, which are plotted in Fig. 3, were then used to estimate the constants A and b using the calibration method outlined in the Theory Section. The average MWs in Table 2 were used in these calculations. In all calibration procedures using one, two, and multiple broad standards, the constants A and b measured in each case are identical to A and b obtained from the narrow MW calibration line. This is to be expected, provided the calibration procedure is valid, because the M_n and M_w values for the broad standards were generated from their respective FlFFF fractograms using the A and b constants obtained using the narrow MW standards calibration line. It

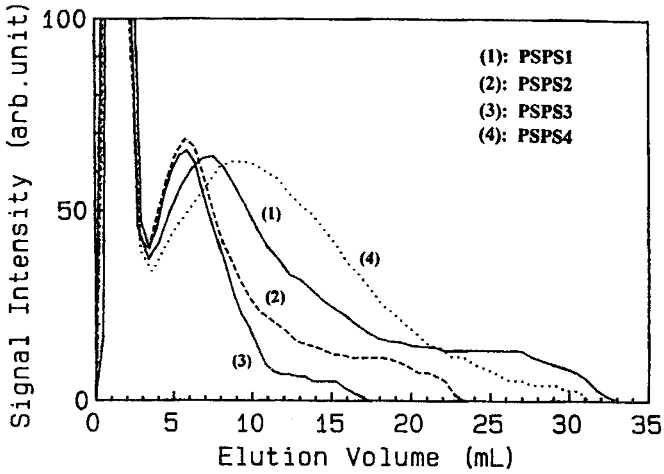

FIG. 3 FlFFF fractograms for four broad PSPS samples, run at conditions indicated in Table 2.

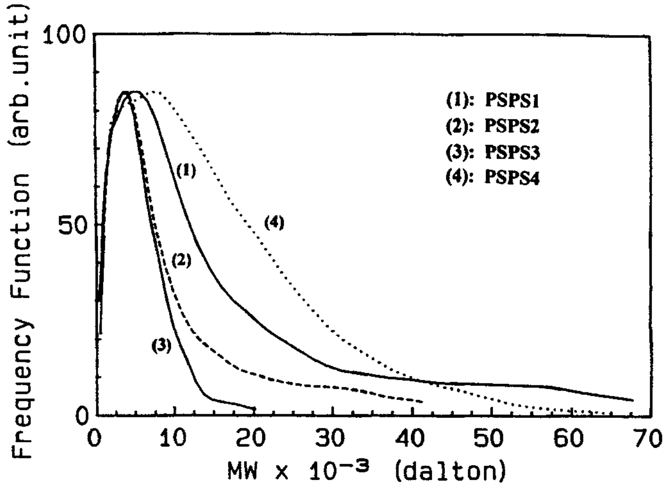

FIG. 4 MWD curves for four broad PSPS samples generated using the fractograms in Fig. 3 and the PSS narrow standard calibration line in Fig. 2.

should be noted that the polydispersities of these four samples are high, between 2.4 to 3.4, as shown in Table 2. The fact that the same values of A and b returned in each calibration procedure not only demonstrates the feasibility of the new calibration technique but also shows that the rounding errors generated from computation are very small.

The digitized fractograms of the narrowly dispersed PSS standards shown in Fig. 1 were also used to estimate the constants A and b using the broad standard calibration method. In contrast to the peak maximum method in which only the M_p value and the corresponding peak maximum retention parameter for each standard were used, in calibration with broad standards the estimations of A and b were carried out using the M_n and M_w values as well as the retention parameter of each digitized point along the fractogram. The calibration was carried out using the various options outlined in the Theory Section using M_n and M_w values for one broad standard, either two M_n or two M_w values for two broad standards, and M_w values for multiple broad standards. In all calibration procedures, M_n and M_w values specified by the manufacturer were employed. Values of constants A and b obtained in each calibration procedure were then used for backcalculation of MW values M_p, M_n, and M_w against PSS standards. The results for A and b from the various methods of estimation and the MW values generated using these constants are listed in Tables 3 to 6.

Tables 3–5 demonstrate how constants A and b were very sensitive to the choice of standards utilized. These constants varied greatly depending on the values of the average MW involved. The values of constants A and b obtained using two M_n values were unrealistic for all combinations of standards (see Table 4).

The use of both M_n and M_w for one broad standard resulted in somewhat improved estimates of A and b and the MW values generated for the four standards. However, only one standard (standard PSS4) out of three gave a deviation from the manufacturer's MW values of less than 20%, as indicated in Table 3.

The use of two M_w values provided a more satisfactory calibration, as shown in Table 5. Although constant A was still rather sensitive to the choice of standard combinations, reasonable MW results were obtained in most cases. The smallest deviations from the manufacturer's MW values of up to 17% were obtained using standards PSS1 and PSS2. The largest deviation up to 35% was observed when standards PSS2 and PSS3 were used. These maximum errors were observed in the M_p values, and the deviations in the M_w and M_n values were usually less than 20%.

The use of multiple standards with their M_w values greatly improved the estimation of A and b, and also the backcalculated MW results compared with the use of just two standards. As shown in Table 6, excellent

TABLE 3

Comparison of M_p, M_n, and M_w Values (dalton) for the Four PSS Standards (PSS1–PSS4) Given by the Manufacturer and Those Obtained from FIFFF Using One Standard for Calibration. Note that M_p from the Manufacturer Refers to the SEC Chromatogram Peak Whereas the Calculated Value Is for the FIFFF Fractogram. The Constants A and b Obtained in Each Case Are Included in the Corresponding Column Heading. The Numbers in Parentheses Indicate the Percentage Deviation from the Manufacturer's Values

Manufacturer MW value	Standard PSS2 $A = 115 \times 10^{-3}$ $b = 0.910$	Standard PSS3 $A = 52.9 \times 10^{-3}$ $b = 0.817$	Standard PSS4 $A = 9.08 \times 10^{-3}$ $b = 0.613$
M_p:			
1,370	2,000 (+46%)	1,840 (+34%)	1,260 (−8.1%)
4,800	4,250 (−11%)	4,260 (−11%)	3,850 (−20%)
6,200	5,720 (−7.7%)	5,930 (−4.3%)	5,980 (−3.5%)
18,500	10,600 (−43%)	11,800 (−36%)	14,900 (−19%)
M_n:			
—	1,530	1,320	694
4,100	4,100 (0%)	4,060 (−1.0%)	3,510 (−14%)
5,900	5,720 (−3.0%)	5,900 (0.0%)	5,800 (−1.7%)
16,000	11,200 (−30%)	12,500 (−22%)	16,000 (0%)
M_w:			
1,430	2,110 (+47%)	1,980 (+38%)	1,460 (+1.9%)
4,500	4,500 (0%)	4,560 (+1.3%)	4,310 (−4.2%)
6,500	6,200 (−4.8%)	6,500 (0.0%)	6,890 (+6.0%)
17,500	11,700 (−33%)	13,200 (−25%)	17,500 (0%)

agreement between A and b values obtained from the peak maximum method and those obtained from the new calibration method using multiple M_w values was observed. Consequently, the MW results generated by both calibration procedures were also very compatible.

TABLE 4

Comparison of Calibration Constants A and b Obtained by FIFFF Using Fractograms and M_n Values (from manufacturer) for Two PSS Calibration Standards in Various Combinations

Standard number	M_n1 (dalton)	M_n2 (dalton)	A ($\times 10^3$)	b
PSS2 and PSS3	4,100	5,900	0.247	0.121
PSS3 and PSS4	5,900	16,000	0.221	0.160
PSS2 and PSS3	4,100	16,000	0.240	0.115

TABLE 5

Comparison of M_p, M_n, and M_w Values (dalton) for the Four PSS Standards (PSS1–PSS4) Given by the Manufacturer and Those Obtained from FlFFF Using Two Standards (M_w values). The Calibration Constants A and b Used in Each Case Are Indicated in the Corresponding Column Heading. The Numbers in Parentheses Indicate the Percentage Deviation from the Manufacturer's Values

Manufacturer	PSS1 and PSS2 $A = 6.80 \times 10^{-3}$ $b = 0.580$	PSS1 and PSS3 $A = 10.2 \times 10^{-3}$ $b = 0.630$	PSS1 and PSS4 $A = 8.60 \times 10^{-3}$ $b = 0.608$	PPS2 and PPS3 $A = 40.0 \times 10^{-3}$ $b = 0.785$	PPS2 and PPS4 $A = 11.0 \times 10^{-3}$ $b = 0.633$	PPS3 and PPS4 $A = 6.28 \times 10^{-3}$ $b = 0.576$
M_p:						
1,370	1,210 (−12%)	1,250 (−9.0%)	1,230 (−10%)	1,750 (+27%)	1,370 (0%)	1,060 (−23%)
4,800	3,970 (−17%)	3,700 (−23%)	3,800 (−21%)	4,180 (−13%)	4,050 (−16%)	3,480 (−27%)
6,200	6,340 (+2.3%)	5,680 (−8.4%)	5,920 (−4.4%)	5,900 (−4.8%)	6,200 (0%)	5,560 (−10%)
18,500	16,770 (−9.3%)	13,810 (−25%)	14,900 (−19%)	12,040 (−35%)	15,020 (−19%)	14,730 (−20%)
M_n:						
—	612	711	670	1,220	785	536
4,100	3,560 (−13%)	3,390 (−17%)	3,450 (−16%)	3,970 (−3.2%)	3,710 (−9.6%)	3,120 (−24%)
5,900	6,090 (+3.2%)	5,520 (−6.4%)	5,730 (−2.8%)	5,850 (−0.8%)	6,030 (+2.2%)	5,340 (−9.4%)
16,000	18,000 (+12%)	14,800 (−7.5%)	15,980 (−0.1%)	12,830 (−20%)	16,090 (+0.6%)	15,810 (−1.2%)
M_w:						
1,430	1,430 (0%)	1,430 (0%)	1,430 (0%)	1,890 (+32%)	1,570 (+9.7%)	1,250 (−12%)
4,500	4,500 (0%)	4,120 (−8.5%)	4,260 (−5.4%)	4,500 (0%)	4,500 (0%)	3,950 (−12%)
6,500	7,400 (+14%)	6,500 (0%)	6,830 (+5.1%)	6,500 (0%)	7,090 (+9.0%)	6,500 (0%)
17,500	19,930 (+14%)	16,110 (−7.9%)	17,500 (0%)	13,550 (−23%)	17,500 (0%)	17,500 (0%)

TABLE 6

Comparison of M_p, M_n, and M_w Values (dalton) for the Four PSS Standards (PSS1–PSS4) Given by the Manufacturer and Those Obtained from FlFFF Using Either the Conventional Calibration or the Multiple Fractogram Method Utilizing the M_w Values. The Calibration Constants A and b Used in Each Case are Shown in the Corresponding Column Heading. The Numbers in Parentheses Indicate the Percentage Deviation from the Manufacturer's Values

Manufacturer	Narrow standard $A = 7.92 \times 10^{-3}$ $b = 0.587$	Broad standard $A = 8.30 \times 10^{-3}$ $b = 0.604$
M_p:		
1,370	1,370 (0%)	1,210 (−12%)
4,800	4,390 (−8.5%)	3,760 (−22%)
6,200	6,960 (+12%)	5,870 (−5.3%)
18,500	18,100 (−2.4%)	14,800 (−20%)
M_n:		
—	712	653
4,100	3,960 (−3.4%)	3,410 (−17%)
5,900	6,700 (+14%)	5,680 (−3.7%)
16,000	19,400 (+21%)	15,900 (−0.5%)
M_w:		
1,430	1,610 (+12%)	1,400 (−1.7%)
4,500	4,960 (+10%)	4,210 (−6.3%)
6,500	8,090 (+24%)	6,780 (+4.3%)
17,500	21,400 (+22%)	17,500 (0%)

CONCLUSION

Determination of MWD is an important application of FlFFF. The conventional method used for this measurement involves constructing a calibration line based on narrow MW standards of known MW. In practice, the lack of such standards for many polymers has limited the utility of FlFFF. An alternative calibration method has been developed and tested with some narrow PSS standards dissolved in Tris buffer maintained at pH8. The theory developed in a companion paper for ThFFF was modified to adapt to FlFFF data. Broad MW PSPS samples, whose MW averages were determined based on FlFFF calibration using narrow PSS standards, were tried first to ensure the new calibration method was feasible with broad standards.

Narrowly dispersed PSS standards were then used with both the peak maximum calibration using M_p values and calibration utilizing the fractograms as if they were broad standards using the manufacturer's M_n and M_w values. In the latter method, retention of all digitized points along each fractogram was taken into account. Various permutations of the method were tried, including calibration using M_n and M_w values for one standard, using either two M_n values or two M_w values for two standards, and using M_w values for multiple standards. Constants A and b obtained in each case were used to generate MW values (M_p, M_n, and M_w) for each standard.

Similar to ThFFF, the use of M_n for calibration is not reliable. This can be explained as due to the errors often associated with M_n values. These errors may arise due to the presence of low MW impurities in the sample and/or to errors in the FlFFF fractogram data generally arising from the poor resolution of the sample in the low retention region near to the void peak.

The use of two M_w values gave quite reasonable results, with the deviations depending on the choice of standards used. The differences between the reported MW and values generated by this method are generally less than 20%. The use of M_w for multiple broad standards greatly reduced these differences. Results from this latter calibration were very compatible to those obtained using the conventional peak maximum method with monodisperse standards.

In conclusion, a new method for calibration using broad standards for FlFFF was developed and tested, and its feasibility using M_w values was confirmed. The method should enable FlFFF to be used to analyze a wider range of polymers. It should be noted that water-soluble polymers were used as the test system in this study. However, the calibration method should be equally applicable to organic-soluble polymers.

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